

LISTING OF THE CLAIMS

1. - 2. (Canceled)

3. (Previously presented) The method of claim 40, wherein the retention layer comprises a particulate material.

4. (Previously presented) The method of claim 3, wherein the particulate material of the retention layer consists of glass beads.

5. (Previously presented) The method of claim 40, wherein the retention layer comprises a rigid retention material.

6. (Previously presented) The method of claim 5, wherein the rigid retention material comprises sinter plates.

7. (Previously presented) The method of claim 40, wherein the clarification reactor has a top and a bottom, wherein the retention layer is disposed inside the clarification reactor between the top and the bottom of the clarification reactor,

wherein the mixture comprising the precipitate and the lysate enters the top of the clarification reactor, and the lysate exits the bottom of the clarification reactor, with the precipitate being retained within the clarification reactor by the retention layer,

wherein in step d), increasing pressure is applied at the top of the clarification reactor to the mixture comprising the precipitate and the lysate, thereby ensuring a constant outflow of the lysate from the bottom of the clarification reactor.

8. (Previously presented) The method of claim 48, wherein the applied pressurized gas is pressurized air.

9. (Previously presented) The method of claim 40, wherein one or more wash steps are inserted between steps d) and e).

10. (Canceled)

11. (Previously presented) The method of claim 40, wherein the flow of the cell suspension and the flow of the lysis solution are combined, without further mixing, before entering the lysis reactor, thus forming a single flow within the lysis reactor that is homogeneously mixed when flowing through the filling elements in the lysis reactor.

12. (Previously presented) The method of claim 40, wherein the cell suspension and the lysis solution are introduced into the lysis reactor in the form of two independent flows.

13. (Currently amended) The method of claim ~~40~~40, wherein the neutralization reactor includes a T-type or a Y-type connector and a tubing system fluidly connecting the lysis reactor to the clarification reactor, the tubing system being in the form of a coil, wherein lysed cell solution and the neutralization solution are combined through the T-type or the Y-type connector, thus forming a single flow through the tubing system, wherein the lysed cell solution and the neutralization solution are mixed to produce the mixture comprising the lysate and the precipitate during transportation through the tubing system between the lysis reactor and the clarification reactor, wherein the tubing system is configured to avoid shearing of flocks of the precipitate.

14. (Currently amended) The method of claim 12, wherein the two flows are transported through the lysis reactor at a defined ratio of flow rates, the flow rates being regulated by pressure or pumps, thereby ensuring a constant ratio of cell suspension flow volumes and lysis solution flow volumes.

15. (Previously presented) The method of claim 40, wherein in step c), the lysed cell solution obtained in step b) is mixed with the neutralization solution in a continuous mode.

16. (Previously presented) The method of claim 15, wherein the lysed cell solution and the neutralization solution are combined at a constant ratio of flow rates.

17. (Previously presented) The method of claim 40, wherein a concentration and/or a conditioning step is inserted between step d) and step e).

18. (Previously presented) The method of claim 17, wherein the concentration step and the condition step are inserted, and wherein said concentration step takes place before said conditioning step.

19. (Currently amended) The method of claim 40, wherein said ~~biomolecule-~~
polynucleotide of interest is a polynucleotide comprising a pDNA, a short linear DNA or RNA.

20. (Currently amended) The method of claim 19, wherein the polynucleotide of interest
is ~~plasmid DNA~~pDNA.

21. - 22. (Canceled)

23. (Currently amended) The method of claim 40 wherein, ~~in addition,~~ step a) is operated
in a continuous mode.

24. (Currently amended) The method of claim 40, wherein the harvested cell suspension
provided in step a) is ~~obtained from a cryo-pelleted cell suspension~~cryopelleted prior to
resuspension.

Claims 25 – 39 (Canceled)

40. (Currently amended) A method of purifying a ~~biomolecule-~~
polynucleotide of interest
from host cells comprising the polynucleotide of interest using an automated or semi-automated

device, wherein the device comprises a lysis reactor, a neutralization reactor and a clarification reactor fluidly connected to one another, the method comprising:

- a) providing a cell suspension of the host cells that have been cultivated to produce the polynucleotide ~~a biomolecule~~ of interest, wherein the cell suspension is a fermentation broth within which the host cells were cultivated or a re-suspension of the cultivated host cells that were harvested from the fermentation broth;
- b) introducing a flow of the cell suspension and a flow of a lysis solution ~~into the lysis reactor~~ into the lysis reactor, the lysis reactor containing filling elements made of glass, plastic, stainless steel or fibrous material, such that the flow of the cell suspension and the flow of the lysis solution ~~into and through the lysis reactor~~ filling elements provides homogenous mixing of the flows in the absence of shear forces and whereby the cultivated host cells of the flowed suspension are substantially disintegrated by alkaline lysis to produce a lysed cell solution;
- c) neutralizing the lysed cell solution via the neutralization reactor wherein the lysed cell solution is mixed with a neutralization solution to produce a mixture comprising a lysate and a precipitate comprising cellular debris and impurities, and wherein the lysate contains the polynucleotide ~~biomolecule~~ of interest;
- d) introducing the mixture comprising the precipitate and the lysate into the clarification reactor wherein the lysate containing the polynucleotide ~~biomolecule~~ of interest is separated from the precipitate, wherein the mixture comprising the precipitate and the lysate is allowed to flow through the clarification reactor, and wherein the clarification reactor contains a retention layer that functions to retain the precipitate but allow the lysate to flow ~~from out of~~ from out of the clarification reactor; and
- e) purifying the polynucleotide ~~biomolecule~~ of interest, where the polynucleotide ~~biomolecule~~ of interest is purified from the lysate that flows ~~from out of~~ from out of the clarification reactor,

wherein said method is operated on a manufacturing scale.

41. (Currently amended) The method of claim 40, wherein one or more distribution means are disposed inside the clarification reactor and extend ~~to onto~~ onto a surface of the retention

layer, wherein the one or more distribution means evenly distribute the mixture comprising the precipitate and the lysate as obtained in step c) into the clarification reactor of step d).

42. (Previously presented) The method of claim 40, wherein the filling elements are a particulate material.

43. (Currently amended) The method of claim 7, wherein the retention layer has a top facing the top of the clarification reactor and a bottom facing the bottom of the clarification reactor, wherein the retention layer functions to retain the precipitate on the top of and within the retention material while allowing the purified lysate to flow ~~from~~ out of the clarification reactor.

44. (Previously presented) The method of claim 42, wherein the particulate material consists of beads, each bead having a diameter in the range of about 1 to about 100 μ m.

45. (Previously presented) The method of claim 44, wherein each bead has the same diameter.

46. (Previously presented) The method of claim 42, wherein the lysis reactor is essentially completely filled with the particulate material.

47. (Currently amended) The method of claim 42, wherein ~~in~~ the particulate material consists of glass beads.

48. (Previously presented) The method of claim 7, wherein pressure is increased by applying pressurized gas.

49. (Currently amended) The method of claim 40, wherein the method is operated to process more than 100 grams wet cells and ~~yield~~ yields from 0.1 g to ~~a~~ 1.0 kilogram of the polynucleotide biomolecule of interest, depending on the amount of wet cell paste processed and the amount of the polynucleotide of interest present in the cells.

50. (Canceled)

51. (New) The method of claim 11, wherein the two flows are transported through the lysis reactor at a defined ratio of flow rates, the flow rates being regulated by pressure or pumps, thereby ensuring a constant ratio of cell suspension flow volumes and lysis solution flow volumes.

52. (New) The method according to claim 40, wherein said method is continuously operated on a manufacturing scale.